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THE BIOCHEMISTRY
OF THE MALARIA PARASITE

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OF THE MALARIA PARASITE
THE EFFECTS OF QUININE AND ATABRINE
ON GLYCOLYSIS

A DISSERTATION SUBMITTED TO THE FACULTY OF THE
DIVISION OF BIOLOGICAL SCIENCES IN CANDIDACY
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BIOCHEMISTRY

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GLYCOLYSIS IN CELL-FREE PREPARATIONS OF THE MALARIA PARASITE

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The formation of lactic acid from glucose is an important reaction in the carbohydrate metabolism of the malaria parasite. Lactic acid appears to be the sole product of glucose utilization by the parasite under anaerobic conditions and also accumulates, as an intermediate, in the oxidation of glucose to carbon dioxide and water in the presence of oxygen (1, 2).

The present report comprises a study of the mechanism of lactic acid formation from glucose by the malaria parasite *Plasmodium gallinaceum*. It has been possible to prepare, for the first time, cell-free enzyme extracts from the parasite capable of the *in vitro* conversion of glucose to lactic acid. These extracts were found to phosphorylate glucose by a transfer of phosphate from adenosine triphosphate and to catalyze the oxidation-reduction between 3-phosphoglyceraldehyde and pyruvic acid.

Methods and Materials

Inorganic phosphate was determined by the method of Gomori (3) on trichloroacetic acid filtrates.

Adenosine triphosphate was isolated from rabbit muscle according to Needham (4). Fructose-1,6-diphosphate was separated from the products of yeast fermentation as the barium salt and purified as the acid barium salt (5). Diphosphopyridine nucleotide was isolated from yeast by the method of Williamson and Green (6).

Other matters of experimental procedure are described later.

Enzyme Preparations

Two types of enzyme preparations were employed in studying the glycolysis of *Plasmodium gallinaceum*. The first type, called a hemolysate, was prepared by treating parasitized red blood cells with water and then centrifuging off the cellular material. This procedure completely laked

* The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Chicago. This work was also supported in part by grants from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

the red cells and partially disrupted the parasites also. Therefore, the hemolysate contained the glycolytic enzymes from both the red cells and the parasites. Although the greater part of the total enzyme activity was due to the enzymes from the parasites, it was necessary to run control experiments with hemolysates of normal red cells. The second type of preparation, called an extract, was made by laking or saponizing parasitized red cells, washing the liberated parasites free from hemoglobin, and then grinding this parasite material and extracting the enzymes. Since the washing removed the glycolytic enzymes of the red cells, control experiments with preparations from normal red blood cells were not necessary.

The red blood cells of chickens infected with *Plasmodium gallinaceum*, strain 8-A, as designated by the Committee on Terminology of Strains of Avian Malaria of the American Society of Parasitologists, were used in making enzyme preparations.¹ Blood was drawn by heart puncture at the height of parasitemia, about 4 days after inoculation of the chickens with infected blood. At this time 60 to 90 per cent of the red cells contained one or more parasites. Clotting was prevented by adding 1/10 volume of 10 per cent sodium citrate to the blood.

Hemolysates—Hemolysates prepared in a manner similar to that described by Meyerhof (7) in his work on glycolysis in erythrocytes were used to study the phosphorylation of glucose. The citrated blood was centrifuged, and the cells were washed three times by suspending them in 4 volumes of 0.9 per cent sodium chloride and centrifuging again. The washed cells were treated with 2 volumes of distilled water and allowed to hemolyze for 15 minutes with occasional shaking. Then sufficient 7 per cent sodium chloride (1/10 volume) was added to restore the salt concentration to about 0.9 per cent, and the solid material was centrifuged off vigorously. The supernatant solution, which contained large amounts of hemoglobin but was perfectly clear, was used as the enzyme. All operations were carried out in a cold room at 0°. The concentration of the hemolysate was calculated in terms of the volume of packed red blood cells used in its preparation.

Extracts—Extracts of parasite material were employed in studying the oxidation-reduction reactions. The citrated blood was centrifuged, and the cells were washed twice by suspending them in 4 volumes of calcium-free phosphate-saline (8) containing 0.1 per cent glucose. The cells were then suspended in 4 volumes of the same medium and treated with sufficient 4 per cent saponin in phosphate-saline (1/40 volume) to make the final concentration of saponin about 0.1 per cent. These conditions of saponization are similar to those described by Christophers and Fulton (9). After the saponin had been thoroughly mixed with the red cell suspension,

¹ I am greatly indebted to Mr. William Cantrell of the Department of Bacteriology and Parasitology for providing the infected birds.

the solid material was centrifuged down and washed twice by suspending in 4 volumes of phosphate-saline-glucose and twice in 0.9 per cent sodium chloride containing 0.1 per cent glucose. This washing removed nearly all the hemoglobin and therefore probably removed the extranuclear enzymes of the red cells also. The washed parasite material was dark gray-brown in color and viscous in consistency; it contained parasites and some white cells and red cell nuclei. This material was ground with an equal volume of powdered Pyrex glass in a mortar or in a bacterial mill (10). 1 volume of water was added to the paste, and after vigorous centrifugation a brown, turbid, cell-free extract was obtained. All steps of the preparation were carried out in a cold room at 0°.

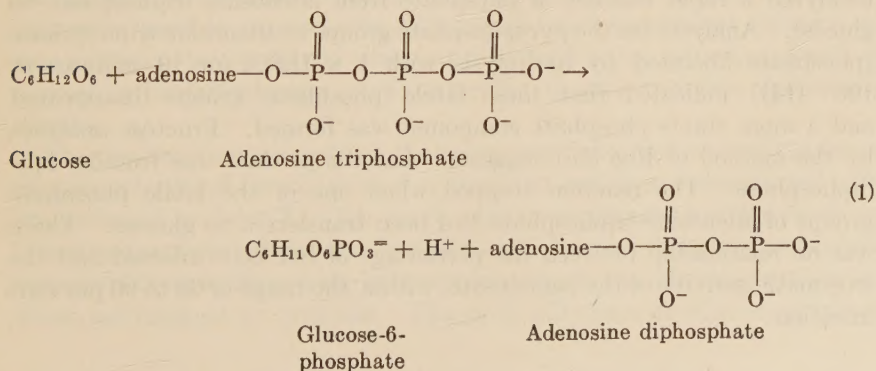
The parasites may also be released from the red blood cells by hemolysis. The procedure used was that described above, except that the washings were carried out with phosphate-saline-glucose and only 1½ volumes of water were used for the hemolysis. This procedure certainly caused some cytolysis of the parasites, since the clear supernatant obtained by centrifuging down the hemolyzed cells was more active in phosphorylating glucose than similar hemolysates prepared from normal chicken red cells. Still, if the solid residue was washed free from hemoglobin, ground with powdered glass, and extracted with water, a solution of enzymes capable of catalyzing the oxidation-reduction reactions was obtained.

Omission of glucose from the wash solutions usually gave extracts of low enzymatic activity. Loss of activity was rapid even at 0°. Extracts prepared in the same way from unparasitized red cells always showed less than 10 per cent of the enzymatic activity usually found in extracts made from parasitized red cells.

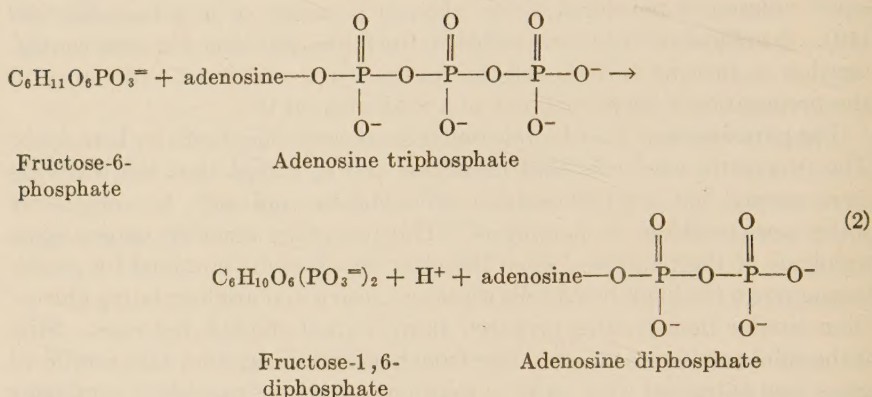
Results

Phosphorylation of Glucose

In yeast glucose is phosphorylated by a transfer of phosphate from adenosine triphosphate to glucose, with the formation of glucose-6-phosphate (11) (Equation 1). This reaction is catalyzed by the enzyme hexokinase.



Most of the subsequent steps in the glycolytic breakdown of glucose-6-phosphate are the same in yeast and in muscle. The enzyme phosphohexoisomerase brings about the rearrangement of glucose-6-phosphate to fructose-6-phosphate (12). In the presence of the enzyme phosphohexokinase, fructose-6-phosphate is converted to fructose-1,6-diphosphate by a second transfer of phosphate from adenosine triphosphate (13) (Equation 2).



Magnesium or manganous ions are necessary as cofactors for these phosphate transfer reactions.

Reactions of Glucose Phosphorylation—The phosphorylation of glucose by the malaria parasite was studied in hemolysates of parasitized red blood cells, and control experiments were carried out with hemolysates of normal chicken red cells. Qualitatively the reactions occurring were the same in hemolysates from both normal and parasitized red cells. Both preparations contained the enzyme adenosinetriphosphatase, which hydrolyzes adenosine triphosphate to adenosine diphosphate or adenylic acid and inorganic phosphate. This enzyme could largely be inhibited by the addition of fluoride. In the presence of fluoride and magnesium ions, the hemolysates catalyzed a rapid transfer of phosphate from adenosine triphosphate to glucose. Analyses for the pyrophosphate groups of adenosine triphosphate (phosphate liberated by hydrolysis with 1 N H_2SO_4 for 10 minutes at 100° (14)) indicated that these labile phosphate groups disappeared and a more stable phosphate compound was formed. Fructose analyses by the method of Roe (15) suggested that the product was fructose-1,6-diphosphate. The reaction stopped when one of the labile phosphate groups of adenosine triphosphate had been transferred to glucose. There was no relationship between the percentage of red cells infected and the enzymatic activity of the hemolysate, within the range of 60 to 90 per cent infection.

Manometric Experiments on Glucose Phosphorylation—As Equations 1 and 2 indicate, the transfer of phosphate from adenosine triphosphate to a hydroxyl group results in the formation of acid. Therefore this reaction can conveniently be studied by employing a bicarbonate buffer in the test system and measuring the evolution of carbon dioxide by the Warburg manometric technique (11). In the case of red blood cell hemolysates, acid formation which occurred in the absence of glucose was the result of hydrolysis of the adenosine triphosphate and is referred to as "adenosinetriphosphatase activity." Acid formation which occurred in the presence of glucose was the result of transfer of phosphate from the adenosine triphosphate to glucose, along with some hydrolysis of the adenosine triphosphate;

TABLE I

Adenosinetriphosphatase and Hexokinase Activity in Red Blood Cell Hemolysates

The samples contained 0.028 M NaHCO_3 , 0.004 M MgSO_4 , 0.028 M KF, 0.012 M glucose (none when adenosinetriphosphatase activity was being measured), 0.0036 M adenosine triphosphate (tipped in from side arm after equilibration), and 1.4 cc. of hemolysate of normal chicken red cells (equivalent to about 0.36 cc. of cells) or 0.7 cc. of hemolysate of parasitized red cells (equivalent to about 0.21 cc. of cells), in a total volume of 2.5 cc. Warburg manometers; gas phase, 5 per cent CO_2 -95 per cent N_2 ; temperature 39°. The retention factor ((observed CO_2)/(true CO_2)) was about 0.7 for mixtures with hemolysates of normal red cells and 0.85 for mixtures with hemolysates of parasitized red cells. Activities are expressed in microliters of CO_2 evolved per cc. of red cells per hour, corrected for retention. The figures in parentheses show the range of values.

Normal red cells			Parasitized red cells		
No. of samples	Adenosine-triphosphatase	Hexokinase	No. of samples	Adenosine-triphosphatase	Hexokinase
6	56 (31-103)	378 (290-591)	12	76 (44-102)	1630 (890-2270)

it is termed "hexokinase activity." Since the hemolysates contained hemoglobin in high concentrations and since different volumes of hemolysate were used in experiments with normal and parasitized material, it was necessary to correct the observed gas evolution for retention of carbon dioxide by the buffering action of the protein, in order to obtain comparable results.

The results of experiments on the adenosinetriphosphatase and the hexokinase activities of hemolysates of normal and parasitized chicken red cells are collected in Table I. The much greater hexokinase activity found in hemolysates of parasitized erythrocytes indicates that the parasites contain considerable quantities of the enzymes hexokinase and phosphohexokinase, which are released by cytolysis. The small difference in adenosinetriphos-

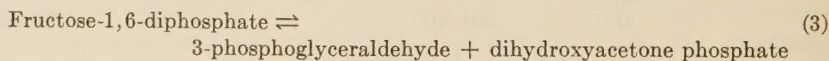
phatase activity between hemolysates of normal and parasitized red cells shows that most of this enzyme present in the hemolysates was derived from the red cells and not from the parasites.

Glucose Phosphorylation in Parasite Extracts—Extracts of parasite material, when tested in the manometric system for studying the phosphorylation of glucose, showed little activity aside from that caused by the enzyme adenosinetriphosphatase. This interfering enzyme was not inhibited by fluoride. Nevertheless, by the use of a different technique it was possible to show that the extracts were able to phosphorylate glucose. This reaction results in the formation of compounds giving a positive test for fructose in the Seliwanoff reaction (fructose-6-phosphate and fructose-1,6-diphosphate). Extracts of parasite material were able to convert glucose to "fructose," and the reaction could be followed by quantitative determinations of fructose made according to Roe (15). Since this conversion was greatly accelerated by the addition of adenosine triphosphate, it undoubtedly involved phosphorylation of glucose by the reactions more clearly demonstrated by the use of hemolysates.

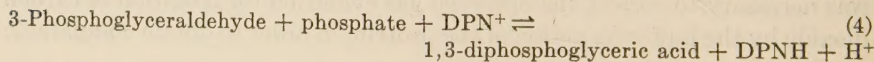
These experiments show that the malaria parasite initiates the breakdown of glucose by phosphorylation in the manner known to occur in yeast extracts.

Oxidation-Reduction Reactions

Muscle and yeast extracts contain an enzyme, aldolase, which catalyzes the splitting of fructose-1,6-diphosphate, formed by the reactions described above, into 2 molecules of triose phosphate, 3-phosphoglyceraldehyde and dihydroxyacetone phosphate (16, 17) (Equation 3). The next step in glycolysis in these extracts is the oxidation of 3-phosphoglyceraldehyde



by diphosphopyridine nucleotide (DPN) in the manner represented by Equation 4 (18); the enzyme catalyzing this reaction is 3-phosphoglyceraldehyde dehydrogenase. This equilibrium reaction goes in the forward

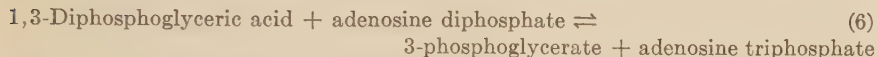


direction because of the further reaction of the 1,3-diphosphoglyceric acid and because of the reoxidation of the reduced DPN (DPNH) by pyruvate (formed from the 1,3-diphosphoglyceric acid) (Equation 5); the enzyme which catalyzes this oxidation, lactic dehydrogenase, is present in muscle extracts. One phosphate group of the 1,3-diphosphoglyceric

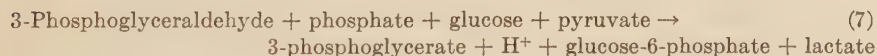
acid is transferred to adenosine diphosphate in the presence of the appro-



priate enzyme (18, 19) (Equation 6). The 1,3-diphosphoglyceric acid



also breaks down spontaneously to 3-phosphoglyceric acid and inorganic phosphate, but the rate of this reaction is slower than that of the enzymatic phosphate transfer. In order that the transfer of phosphate from 1,3-diphosphoglyceric acid to adenosine diphosphate may proceed nearly to completion, adenosine diphosphate must be present in excess or must be continuously regenerated from the adenosine triphosphate. Adenosine diphosphate may be formed from adenosine triphosphate as the result of hydrolysis by the enzyme adenosinetriphosphatase or as the result of a transfer of one phosphate group from adenosine triphosphate to a phosphate acceptor such as glucose (Equation 1). Magnesium ions are required as a cofactor for these phosphate transfer reactions. On the basis of these facts, one suitable system for studying the oxidation of 3-phosphoglyceraldehyde would contain stoichiometric amounts of the substrate, inorganic phosphate, glucose, and pyruvate, and catalytic amounts of DPN, adenosine diphosphate (or adenosine triphosphate), and magnesium ions. The over-all reaction occurring in this system (the sum of Equations 4, 5, 6, and 1) is represented by Equation 7. It was



felt that the demonstration of such an oxidation in parasite material would be strong evidence for the occurrence of a phosphorylating glycolysis in the malaria parasite.

Aldolase Reaction—Extracts of parasite material were able to split fructose-1,6-diphosphate to triose phosphate. The products of the splitting of fructose-1,6-diphosphate by the enzyme aldolase, 3-phosphoglyceraldehyde and dihydroxyacetone phosphate, may be identified by the fact that their phosphate groups are readily hydrolyzed by N NaOH at room temperature (16). Since the reaction reaches an equilibrium unless the products are removed, cyanide was added to bind the triose phosphate. In a system containing fructose-1,6-diphosphate, cyanide, and an extract of parasite material, the formation of compounds containing alkali-labile phosphate groups was observed. This indicates that the fructose-1,6-diphosphate was being split in the manner represented by Equation 3.

Coupled Oxidation-Reduction Reaction—The oxidation of 3-phosphoglyceraldehyde according to Equation 7 results in the formation of acid. Therefore the process can be followed conveniently by measuring the carbon dioxide liberated from a bicarbonate buffer containing the test system. The test system used in studying the oxidation contained magnesium, fluoride, inorganic phosphate, glucose, fructose-1,6-diphosphate, pyruvate, DPN, adenosine triphosphate, and an extract of parasite material. Fluoride was added to inhibit partially the adenosinetriphosphatase present in the extracts and to prevent the breakdown of the 3-phosphoglycerate to pyruvate (20, 21). Fructose-1,6-diphosphate was used as the substrate, since the extracts contained the enzyme aldolase.

TABLE II

Components of System Oxidizing 3-Phosphoglyceraldehyde

The complete system contained 0.026 M NaHCO_3 , 0.004 M MgSO_4 , 0.008 M phosphate at pH 7.4, 0.02 M KF, 0.013 M glucose, 0.013 M sodium pyruvate, 0.0007 M adenosine triphosphate, 0.00007 M diphosphopyridine nucleotide, 0.0035 M fructose-1,6-diphosphate (tipped in from the side arm), and 1.7 cc. of an extract of parasite material (prepared by saponization, 0.9 per cent NaCl without glucose used for all washings), in a total volume of 2.5 cc. Warburg manometers; gas phase, 5 per cent CO_2 -95 per cent N_2 ; temperature 39° .

Sample	Rate
	<i>microliters CO_2 per hr.</i>
Complete system.....	56
Without fructose-1,6-diphosphate.....	44
" glucose.....	42
" adenosine triphosphate.....	41
" phosphate.....	39
" MgSO_4	34
" diphosphopyridine nucleotide.....	33
" pyruvate.....	27

Oxidation of 3-phosphoglyceraldehyde by extracts of parasite material required all the components of this test system, as shown by the data in Table II. Omission of fructose-1,6-diphosphate, glucose, adenosine triphosphate, inorganic phosphate, magnesium, DPN, or pyruvate decreased the rate of the reaction by 20 to 50 per cent. The activity of the system in this experiment was much smaller than that usually obtained, because in the preparation of the extract it was necessary to omit glucose from the wash solutions.

Effects of Arsenate and Iodoacetate—Arsenate and iodoacetate have characteristic effects on the oxidation of 3-phosphoglyceraldehyde. Arsenate is believed to replace inorganic phosphate in Reaction 4, forming

1-arseno-3-phosphoglyceric acid as the product of the oxidation; this substance breaks down rapidly and spontaneously to arsenate and 3-phosphoglyceric acid (18). Therefore, in the presence of arsenate the oxidation of 3-phosphoglyceraldehyde occurs according to Equation 8. The coupling

3-Phosphoglyceraldehyde + DPN⁺ → 3-phosphoglycerate + DPNH + 2 H⁺ (8)
of the oxidation with the uptake and transfer of phosphate is avoided.

TABLE III

Effect of Arsenate and Iodoacetate on Oxidation of 3-Phosphoglyceraldehyde

The complete system contained 0.026 M NaHCO₃, 0.004 M MgSO₄, 0.02 M KF, 0.0035 M fructose-1,6-diphosphate, 0.013 M sodium pyruvate, 0.00007 M diphosphopyridine nucleotide, 0.008 M phosphate at pH 7.4, 0.013 M glucose, 0.0007 M adenosine triphosphate, and 1.7 cc. of extract of parasite material, in a total volume of 2.5 cc. The arsenate sample contained 0.0012 M Na₂HAsO₄ and no phosphate, glucose, or adenosine triphosphate. The iodoacetate sample was like the arsenate sample with the addition of 0.0012 M sodium iodoacetate. Warburg manometers; gas phase, 5 per cent CO₂-95 per cent N₂; temperature 39°.

Sample	Rate <i>microliters CO₂ per hr.</i>
Complete.....	143
Arsenate.....	221
Iodoacetate.....	19

TABLE IV

Lactic Dehydrogenase Activity of Parasite Extracts

The complete system contained 0.02 M phosphate at pH 7.4, 54 γ of sodium dichlorophenol indophenol, 0.1 M lithium *dl*-lactate, 0.00003 M diphosphopyridine nucleotide, and 0.4 cc. of extract, in a total volume of 5.0 cc. The parasite material was prepared by hemolyzing the red cells, and the extract was dialyzed against 0.9 per cent NaCl for 4 hours at 0°. Temperature 21°. ΔG represents the increase in percentage transmission, measured in an Evelyn photoelectric colorimeter with Filter 620.

Sample	Rate, ΔG in 4 min.
Complete.....	10.2
Without lactate.....	4.0
“ diphosphopyridine nucleotide.....	4.2

With arsenate present instead of inorganic phosphate, Reaction 7 would be changed to Reaction 9. Since the phosphate transfer reactions are

3-Phosphoglyceraldehyde + pyruvate → 3-phosphoglycerate + H⁺ + lactate (9)
usually slower than the oxidation itself, the addition of arsenate characteristically increases the rate of the oxidation of 3-phosphoglyceraldehyde and makes the reaction independent of the presence of inorganic phosphate,

magnesium, adenosine triphosphate, and glucose or other phosphate acceptors. The specific action of iodoacetate consists in inhibiting 3-phosphoglyceraldehyde dehydrogenase, the enzyme which catalyzes Reactions 4 and 8 (22). Few other known enzymes are equally sensitive to low concentrations (0.001 M) of iodoacetate.

Table III gives the results of an experiment on the effects of arsenate and iodoacetate on the oxidation of 3-phosphoglyceraldehyde in extracts of parasite material. Arsenate markedly accelerated the reaction, and iodoacetate caused an inhibition of more than 90 per cent.

Lactic Dehydrogenase—The presence in extracts of parasite material of the enzyme lactic dehydrogenase, which catalyzes Reaction 5, was demonstrated by means of the colorimetric technique described by Haas (23). An Evelyn photoelectric colorimeter with Filter 620 was used to measure the rate of reduction of the dye. The change in percentage transmission (ΔG) per unit time was proportional to the enzyme activity, and the rate was constant over short periods of time. The results of an experiment on the lactic dehydrogenase activity of an extract of parasite material are given in Table IV. It is difficult to obtain preparations with no activity in the absence of added DPN or lactate.

The experiments which have just been described indicate that the malaria parasite is capable of converting fructose-1,6-diphosphate to 3-phosphoglyceraldehyde and of oxidizing the latter substance by a mechanism similar to that found in muscle extracts.

DISCUSSION

No previous study has been made of the mechanism of the formation of lactic acid from glucose by protozoa. Therefore it is of interest to find that the malaria parasite glycolyzes by a process similar to that found in a wide variety of other organisms (plants, bacteria, yeast, vertebrates). In this respect, as in the oxidative reactions thus far investigated, the metabolism of the parasite conforms to a pattern common among living cells.

SUMMARY

The preparation of cell-free enzyme extracts from the malaria parasite is described. These extracts convert glucose to lactic acid by a path similar to the phosphorylating glycolysis of yeast and vertebrate muscle. The preparations contain enzymes which catalyze the phosphorylation of glucose by adenosine triphosphate, the splitting of fructose-1,6-diphosphate to form 3-phosphoglyceraldehyde, and the dismutation between 3-phosphoglyceraldehyde and pyruvic acid.

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THE BIOCHEMISTRY OF THE MALARIA PARASITE*

THE EFFECTS OF QUININE AND ATABRINE ON GLYCOLYSIS

By JOHN F. SPECK

In the preceding paper (1) it was shown that the formation of lactic acid from glucose by the malaria parasite is a process similar to the phosphorylating glycolysis of yeast and vertebrate muscle. Quinine and atabrine have been found to inhibit the glycolysis (2) as well as the oxygen uptake (2-4) of the parasite. Since glycolysis is a preliminary stage in the oxidation of glucose under aerobic conditions and is the only path for the utilization of glucose anaerobically, it was believed that an investigation of the effects of antimalarial drugs on this process might aid in understanding the mode of action of these drugs.

The present paper describes the action of quinine and atabrine on the phosphorylation of glucose and the dehydrogenation of 3-phosphoglyceraldehyde and lactic acid by preparations of the avian parasite *Plasmodium gallinaceum*. Comparable experiments with glycolytic enzymes from yeast and mammalian muscle are also reported.

Methods and Materials

Inorganic phosphate was determined by the method of Gomori (5) on trichloroacetic acid filtrates. Quinine interferes with the estimation of phosphate by the molybdenum blue method by forming a precipitate with phosphomolybdic acid, and atabrine interferes because of its deep yellow color. Both difficulties can be avoided by extracting the free bases from alkaline solution with an immiscible solvent. The solution to be analyzed for phosphate is made alkaline to phenolphthalein, extracted twice with an equal volume of carbon tetrachloride, and clarified by centrifugation.

Solutions of quinine and atabrine for use in the enzyme experiments were prepared by neutralizing solutions of the dihydrochlorides with 1.5 equivalents of sodium bicarbonate. The final concentration of the neutralized solutions was not made greater than 0.01 M; otherwise the bases did not remain in solution long enough to permit their being pipetted into the reaction mixtures.

* The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Chicago. This work was also supported in part by grants from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

Adenosine triphosphate, fructose-1,6-diphosphate, and diphosphopyridine nucleotide were prepared as described in the preceding paper (1). The glycogen and adenylic acid were commercial preparations. Potassium glucose-1-phosphate was obtained essentially by the method of Hanes (6). In my experience, after removal of barium with sulfuric acid and neutralization with potassium hydroxide, the potassium glucose-1-phosphate did not precipitate on addition of an equal volume of alcohol. It could be obtained in crystalline form by adding more alcohol to the supernatant and allowing it to stand in the cold. Phosphoglyceric acid was prepared according to Neuberg and Kobel (7). Phosphopyruvic acid was synthesized by a modification of the method of Kiessling (8) suggested by Dr. Gerhardt Schmidt.¹ Adenosine diphosphate was prepared from adenosine triphosphate by the use of yeast hexokinase (9).

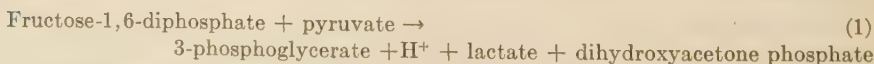
The preparation of enzyme extracts from malaria parasites (*Plasmodium gallinaceum*) is described in the preceding paper (1). The enzymes from yeast and muscle are described in connection with the particular reaction for which they were used.

Results

Effects of Quinine and Atabrine on Glycolysis in Preparations from Malaria Parasites

Phosphorylation of Glucose—The effect of quinine and atabrine on the phosphorylation of glucose by hemolysates of normal and parasitized chicken red blood cells was studied with the manometric system described in the preceding paper (1). The results of several experiments are summarized in Table I. Quinine in concentrations from 0.0001 to 0.002 M inhibited the phosphorylation of glucose in hemolysates of normal chicken red cells but caused only a slight inhibition in hemolysates of parasitized red cells. The inhibition in the latter case was not greater than would be expected from inhibition of that fraction of the total hexokinase activity (about one-fourth) derived from the enzymes of the red cells themselves. However, atabrine in the same concentrations as quinine caused an equal inhibition of the phosphorylation of glucose, in hemolysates of both normal and parasitized erythrocytes.

3-Phosphoglyceraldehyde Dehydrogenase—The effects of quinine and atabrine on the enzyme 3-phosphoglyceraldehyde dehydrogenase were studied in a system in which the over-all process was that described by Equation 1. Three enzymatic reactions are involved in this process:



(1) the splitting of fructose-1,6-diphosphate to 3-phosphoglyceraldehyde

¹ Schmidt, G., personal communication.

and dihydroxyacetone phosphate (catalyzed by the enzyme aldolase); (2) the oxidation of 3-phosphoglyceraldehyde by diphosphopyridine nucleotide in the presence of arsenate to form 3-phosphoglyceric acid and reduced diphosphopyridine nucleotide (catalyzed by the enzyme 3-phosphoglyceraldehyde dehydrogenase); and (3) the reoxidation of the reduced diphosphopyridine nucleotide by pyruvate to form diphosphopyridine nucleotide and lactate (catalyzed by the enzyme lactic dehydrogenase) (see the discussion of the oxidation-reduction reactions in the preceding paper (1)). An excess of purified preparations of the enzymes aldolase and lactic dehydrogenase was added; therefore the rate of the over-all process was determined by the activity of the 3-phosphoglyceraldehyde dehydrogenase.

TABLE I

Effect of Quinine and Atabrine on Hexokinase Activity of Red Blood Cell Hemolysates

The sample contained 0.028 M NaHCO₃, 0.004 M MgSO₄, 0.028 M KF, 0.012 M glucose, 0.0036 M adenosine triphosphate (added from the side arm after equilibration), 1.4 cc. of hemolysate of normal chicken red cells or 0.7 cc. of hemolysate of parasitized red cells, and the concentrations of quinine and atabrine indicated below, in a total volume of 2.5 cc. Warburg manometers; gas phase, 5 per cent CO₂-95 per cent N₂; temperature 39°.

Concentration of inhibitor	Per cent inhibition by			
	Quinine		Atabrine	
	Normal	Parasitized	Normal	Parasitized
M				
0.0001	6	4	6	8
0.0005	18	4	8	18
0.001	20	8	31	26
0.002	43	10	40	39

Aldolase was purified according to Herbert *et al.* (10) through the stage of the first fractionation with ammoniacal ammonium sulfate. Lactic dehydrogenase was prepared from beef heart by following the method of Straub (11) through the second ammonium sulfate precipitation. Since acid is formed, the reaction was followed by measuring the evolution of carbon dioxide from a bicarbonate buffer containing the test system. The complete test system contained bicarbonate, arsenate, fluoride, fructose-1,6-diphosphate, pyruvate, and the three enzymes aldolase, 3-phosphoglyceraldehyde dehydrogenase, and lactic dehydrogenase. If only aldolase and lactic dehydrogenase were added, no acid was formed. Therefore these two preparations were free from 3-phosphoglyceraldehyde dehydrogenase activity. When a preparation containing 3-phosphoglyceraldehyde dehydrogenase was added, in addition to the other two enzymes, acid was formed at a rate which was proportional to the amount of dehydrogenase

added and was constant until at least one-third of the fructose-1,6-diphosphate had been used. Extracts of parasite material (1) as well as preparations made from rabbit skeletal muscle were effective as sources of 3-phosphoglyceraldehyde dehydrogenase.

The effect of quinine and atabrine on the enzyme 3-phosphoglyceraldehyde dehydrogenase extracted from parasite material was investigated by the use of the system just described. In concentrations up to 0.002 M neither drug inhibited this enzyme significantly. These results are shown in Table II.

Lactic Dehydrogenase—The effect of quinine and atabrine on the enzyme lactic dehydrogenase in extracts of parasite material was studied by means

TABLE II
*Effect of Quinine and Atabrine on 3-Phosphoglyceraldehyde
Dehydrogenase in Parasite Extracts*

The samples contained 0.026 M NaHCO_3 , 0.005 M Na_2HAsO_4 , 0.03 M NaF, 0.01 M sodium pyruvate, 0.005 M fructose-1,6-diphosphate (added from side arm after equilibration), 0.00037 M diphosphopyridine nucleotide, aldolase preparation containing 1 mg. of protein, lactic dehydrogenase preparation containing 3 mg. of protein, 0.8 cc. of an extract of parasite material (prepared by hemolysis), and the concentrations of quinine and atabrine indicated below, in a total volume of 2.5 cc. Warburg manometers; gas phase, 5 per cent CO_2 -95 per cent N_2 ; temperature 39°.

Concentration of inhibitor	Activity of dehydrogenase	
	Quinine	Atabrine
M	microliters CO_2 per 5 min.	microliters CO_2 per 5 min.
0	14.6	12.6
0.0001	14.0	12.8
0.0003	14.0	13.2
0.001	14.1	12.5
0.002	14.2	12.9

of the colorimetric technique of Haas (12), as described in the preceding paper (1). The results of two experiments are shown in Table III. Both drugs inhibited the enzyme, but atabrine was considerably more effective than quinine.

Effect of Quinine and Atabrine on Yeast Hexokinase

The enzyme hexokinase in yeast catalyzes the transfer of one phosphate group from adenosine triphosphate to glucose, to form glucose-6-phosphate and adenosine diphosphate (9). This same reaction is apparently the first step in the phosphorylation of glucose by the malaria parasite also (1).

Hexokinase was prepared from bakers' yeast according to Colowick and Kalckar (9), and the reaction was studied manometrically in a test system

similar to theirs. Determination of the labile pyrophosphate groups of adenosine triphosphate (phosphate hydrolyzed by 1 N H_2SO_4 in 10 minutes at 100° (13)) showed that the formation of acid was accompanied by a decrease in pyrophosphate groups and the formation of a more stable phosphate compound.

TABLE III

Effect of Quinine and Atabrine on Lactic Dehydrogenase in Parasite Extracts

The samples contained 0.02 M phosphate at pH 7.4, 54 γ of sodium dichlorophenol indophenol, 0.1 M lithium *dl*-lactate, 0.00003 M diphosphopyridine nucleotide, 0.4 cc. of an extract of parasite material, and the concentrations of quinine and atabrine indicated below, in a total volume of 5.0 cc.; temperature 20° . The rate of reaction was measured as the increase in percentage transmission per unit time, read in an Evelyn photoelectric colorimeter with Filter 620.

Concentration of inhibitor	Per cent inhibition by	
	Quinine	Atabrine
<i>M</i>		
0.0001	5	14
0.0003	7	26
0.001	16	38

TABLE IV

Effect of Quinine and Atabrine on Yeast Hexokinase

The samples contained 0.02 M NaHCO_3 , 0.01 M MgSO_4 , 0.02 M glucose, 0.005 M adenosine triphosphate (tipped in from the side arm after equilibration), hexokinase preparation containing 1.0 mg. of protein, and the concentrations of quinine and atabrine indicated below, in a total volume of 2.0 cc. Warburg manometers; gas phase, 5 per cent CO_2 -95 per cent N_2 ; temperature 30° . The rate of reaction was about 140 microliters of CO_2 per hour.

Concentration of inhibitor	Per cent inhibition by	
	Quinine	Atabrine
<i>M</i>		
0.0005	6	2
0.001	16	8
0.002	23	22

The average results of seven experiments with quinine and the results of a single experiment with atabrine are given in Table IV. Both quinine and atabrine are seen to inhibit the action of yeast hexokinase.

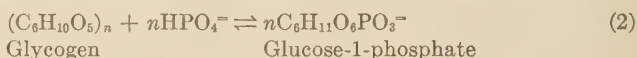
Effect of Quinine on Glycolysis by Muscle Enzymes

Acid Formation from Glycogen—The over-all reaction of glycolysis in rabbit muscle extracts is the formation of lactic acid from glycogen. Since

acid is produced, the course of the reaction can be followed by measuring the evolution of carbon dioxide from a bicarbonate buffer containing the test system (14). The system used contained 0.028 M NaHCO_3 , 0.007 M phosphate at pH 7.4, 9 mg. of glycogen, and 50 mg. of powder obtained by lyophilizing an aqueous extract of rabbit skeletal muscle (14), in a volume of 3.0 cc. The samples were placed in Warburg manometers under an atmosphere of 5 per cent CO_2 -95 per cent N_2 , at a temperature of 35° , and the glycogen was tipped in from the side arm after equilibration. About 300 microliters of CO_2 per hour were evolved.

Quinine consistently inhibited the formation of acid in this system. The average results from several experiments indicated an inhibition of 8 per cent at a quinine concentration of 0.0005 M, of 14 per cent at 0.001 M, and of 31 per cent at 0.002 M. The inhibition was greatest when the enzyme was incubated with quinine before the reaction was started. When the quinine was tipped in at the same time as the glycogen, smaller inhibitions were observed.

Phosphorylase—The phosphorylation of glycogen in muscle extracts occurs in the manner indicated in Equation 2 (15). Phosphorylase, the



enzyme catalyzing this reaction, was prepared from rabbit muscle extract according to Green, Cori, and Cori (16). It was not crystalline but was active and free from phosphoglucomutase activity (conversion of glucose-1-phosphate to glucose-6-phosphate).

The forward reaction, formation of glucose-1-phosphate, was studied in a system containing the enzyme phosphorylase, inorganic phosphate, glycogen, adenylic acid (as coenzyme), and reduced glutathione (to insure maximum activity of the enzyme). The reaction was followed by determining the decrease in inorganic phosphate. The results of experiments on the effect of quinine on the phosphorylation of glycogen are given in Table V. Quinine markedly inhibited this reaction. The inhibition was proportional to the concentration of quinine and decreased with time.

The reverse reaction, formation of polysaccharide from glucose-1-phosphate, was studied in a system like that of Cori and Cori (17). The process was followed by measuring the increase in inorganic phosphate. A marked inhibition by quinine was observed, just as with the forward reaction. The degree of inhibition was proportional to the concentration of quinine and decreased with time. These results are illustrated in Table VI.

Since these experiments measured the rate at which the reactions approached an equilibrium, a decrease in inhibition with longer periods of time was to be expected.

Phosphoglucomutase—The enzyme phosphoglucomutase catalyzes the attainment of an equilibrium between glucose-1-phosphate and glucose-6-phosphate (18). The enzyme used in these experiments was prepared according to Colowick and Sutherland (19), and the test system was similar to theirs.

TABLE V

Effect of Quinine on Phosphorolysis of Glycogen

The samples contained 0.02 M reduced glutathione, 0.001 M adenylic acid, 0.05 M phosphate at pH 7.2, 1 per cent glycogen, phosphorylase preparation containing 6 mg. of protein, and the concentrations of quinine indicated below, in a total volume of 4 cc.; temperature 25°.

Time	Decrease in inorganic P		Per cent inhibition		
	No quinine	0.001 M quinine	Concentration of quinine		
			0.0005 M	0.001 M	0.002 M
min.	γ per cc.	γ per cc.			
20	180	90	37	50	100
40	250	190	19	24	58

TABLE VI

Effect of Quinine on Polysaccharide Formation from Glucose-1-Phosphate

The samples contained 0.05 M glycerophosphate at pH 6.65, 0.02 M reduced glutathione, 0.001 M adenylic acid, 0.5 per cent glycogen, 0.016 M potassium glucose-1-phosphate, phosphorylase preparation containing 4 mg. of protein, and the concentrations of quinine indicated below, in a total volume of 4.0 cc.; temperature 20°.

Time	Increase in inorganic P		Per cent inhibition		
	No quinine	0.001 M quinine	Concentration of quinine		
			0.0005 M	0.001 M	0.002 M
min.	γ per cc.	γ per cc.			
5	132	76	17	42	76
10	252	161	14	36	60

The results of a typical experiment on the effect of quinine on phosphoglucomutase are shown in Table VII. Quinine inhibited the conversion of glucose-1-phosphate to glucose-6-phosphate, but to a variable extent.

Phosphorylation of Fructose-6-phosphate—Fructose-6-phosphate is converted to fructose-1,6-diphosphate by a transfer of phosphate from adenosine triphosphate, catalyzed by the enzyme phosphohexokinase (20). Since this transfer results in the liberation of acid, rate studies can conveniently be made by measuring the evolution of carbon dioxide when the reaction takes place in a bicarbonate buffer. The reaction mixture used

in these experiments contained 0.02 M NaHCO_3 , 0.006 M MgSO_4 , 0.0012 M sodium iodoacetate, 0.012 M potassium glucose-1-phosphate, 0.004 M adenosine triphosphate (tipped in from the side arm after equilibration), and a dialyzed extract equivalent to 80 mg. of an acetone powder of rabbit muscle (21), in a total volume of 2.5 cc. The gas phase in the Warburg manometers was 5 per cent CO_2 -95 per cent N_2 , and the temperature was 37° . The iodoacetate prevented liberation of acid by further reaction of the fructose-1,6-diphosphate formed. Glucose-1-phosphate was used as the substrate instead of fructose-6-phosphate because of its availability. During the preliminary incubation before the adenosine triphosphate was tipped in and during the subsequent experimental period, the glucose-1-phosphate was converted to glucose-6-phosphate and the latter substance rearranged to fructose-6-phosphate by the enzymes phosphoglucumutase and phosphohexoisomerase, which were present in the acetone powder

TABLE VII

Effect of Quinine on Phosphoglucumutase

The samples contained 0.04 M veronal buffer at pH 7.4, 0.005 M MgSO_4 , 0.5 per cent reduced glutathione, 0.0065 M potassium glucose-1-phosphate, enzyme solution containing 12.5 γ of protein, and the concentrations of quinine indicated below, in a total volume of 1.0 cc. Incubated for 5 minutes at 30° .

Quinine concentration	Decrease in glucose-1-phosphate P	Inhibition
M	γ per cc.	per cent
0	127	
0.0005	108	15
0.001	109	14
0.002	73	42

extract. Since the phosphate groups of fructose-6-phosphate and fructose-1,6-diphosphate are less easily hydrolyzable than the pyrophosphate groups of adenosine diphosphate and triphosphate, the reaction can be followed approximately by measuring the decrease in pyrophosphate (phosphate liberated by hydrolysis with 1 N H_2SO_4 for 10 minutes at 100° (13)). Experiments in which organic phosphate fractions were followed showed that acid production was accompanied by a decrease in pyrophosphate groups. Control manometric experiments demonstrated that no acid was formed from adenosine triphosphate in the absence of glucose-1-phosphate, from glucose-1-phosphate in the absence of adenosine triphosphate, or from fructose-1,6-diphosphate. The rate of reaction in the complete system was about 160 microliters of CO_2 in 30 minutes.

Quinine in concentrations from 0.0005 to 0.002 M caused no inhibition of the phosphorylation of fructose-6-phosphate.

Aldolase—The enzyme aldolase catalyzes the splitting of fructose-1,6-

diphosphate to 3-phosphoglyceraldehyde and dihydroxyacetone phosphate. This enzyme was purified according to Herbert *et al.* (10) through the stage of heat coagulation, and the reaction was studied in a test system similar to the one described by these authors. Since the reaction reaches an equilibrium unless the products are removed, cyanide was added to bind the triose phosphate. Samples contained 0.05 M borate buffer at pH 7.3, 0.1 M HCN at pH 7.3, 0.012 M fructose-1,6-diphosphate, and 0.3 cc. of enzyme solution, in a total volume of 4.0 cc. These samples were incubated at 37°. Since the phosphate groups of both molecules of triose phosphate are hydrolyzed by treatment with 1 N NaOH for 20 minutes at room temperature while those of fructose-1,6-diphosphate are not

TABLE VIII

Effect of Quinine and Atabrine on 3-Phosphoglyceraldehyde Dehydrogenase from Rabbit Muscle

The samples contained 0.03 M NaHCO₃, 0.005 M Na₂HAsO₄, 0.03 M NaF, 0.02 M sodium pyruvate, 0.00037 M diphosphopyridine nucleotide, 0.005 M fructose-1,6-diphosphate (tipped in from the side arm after equilibration), aldolase preparation containing 1 mg. of protein, lactic dehydrogenase preparation containing 4 mg. of protein, dialyzed extract equivalent to 5 mg. of acetone powder of rabbit muscle extract (21), and the concentrations of quinine and atabrine indicated below, in a total volume of 2.5 cc. Warburg manometers; gas phase, 5 per cent CO₂-95 per cent N₂; temperature 30°.

Concentration of inhibitor	Activity of dehydrogenase	
	Quinine	Atabrine
M	microliters CO ₂ per 5 min.	microliters CO ₂ per 5 min.
0	10.9	13.7
0.0001	10.7	13.1
0.0003	9.9	12.8
0.001	10.0	12.6
0.002	10.4	12.0

affected, the reaction was followed by determining the increase in alkali-labile phosphate (22). In 5 minutes about 220 γ of alkali-labile P per cc. were formed.

Quinine in concentrations from 0.0005 to 0.002 M caused no significant inhibition of the enzyme aldolase.

3-Phosphoglyceraldehyde Dehydrogenase—The effect of quinine and atabrine on the enzyme 3-phosphoglyceraldehyde dehydrogenase from rabbit muscle was investigated by means of the test system involving Reaction 1, which was used to study the activity of the same enzyme in parasite extracts. As in the latter case neither quinine nor atabrine caused a significant inhibition of 3-phosphoglyceraldehyde dehydrogenase. The results of two experiments are given in Table VIII.

Lactic Dehydrogenase—The action of quinine and atabrine on the enzyme lactic dehydrogenase obtained from beef heart was studied with the aid of the colorimetric technique of Haas (12), as described in Paper II (1). The enzyme was purified by the method of Straub (11) through the stage of the second ammonium sulfate precipitation. In the test system the enzyme showed no activity in the absence of lactate or diphosphopyridine nucleotide.

Lactic dehydrogenase from beef heart was strongly inhibited by atabrine but was not significantly affected by quinine. The experimental data are given in Table IX. The results are similar to those obtained with lactic dehydrogenase from parasite extracts.

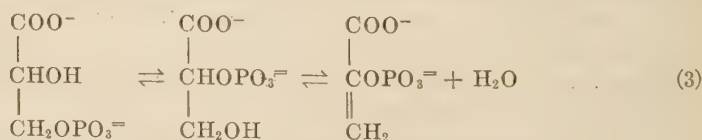
TABLE IX

Effect of Quinine and Atabrine on Lactic Dehydrogenase from Beef Heart

The samples contained 0.03 M phosphate buffer at pH 7.2, 54 γ of sodium dichlorophenol indophenol, 0.1 M lithium *dl*-lactate, 0.00003 M diphosphopyridine nucleotide, lactic dehydrogenase preparation containing 1.1 mg. of protein, and the concentrations of quinine and atabrine indicated below, in a total volume of 5.0 cc.; temperature 25°. The rate of reaction was measured as the increase in percentage transmission (ΔG) per unit time; read in an Evelyn photoelectric colorimeter with Filter 620. Control samples showed a value for ΔG of 16.0 in 4 minutes.

Concentration of inhibitor M	Per cent inhibition by	
	Quinine	Atabrine
0.0001	2	9
0.0003	0	16
0.001	6	41

Rearrangement of 3-Phosphoglycerate to Phosphopyruvate—The first steps in the breakdown of 3-phosphoglycerate in muscle extracts are rearrangement to 2-phosphoglycerate and dehydration of the latter substance to phosphopyruvate (23, 24) (Equation 3). The conversion of 3-phosphoglyc-

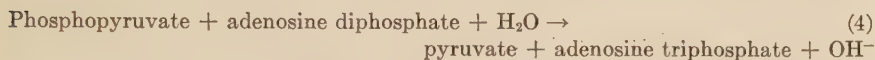


erate to phosphopyruvate can be followed by determining the increase in phosphopyruvate phosphate, which is released on oxidation of the phosphopyruvate by hypiodite (23). The reaction mixture used in studying this process contained 0.05 M veronal buffer at pH 8.2, 0.005 M MgSO_4 , 0.016 M 3-phosphoglycerate, and dialyzed extract equivalent to 4 or 5 mg. of

acetone powder of rabbit muscle (21), in a total volume of 1.0 cc. The samples were incubated for 10 minutes at 37°. Control samples formed about 125 γ of phosphopyruvate P per cc.

Quinine in concentrations from 0.0002 to 0.002 M did not inhibit the conversion of 3-phosphoglycerate to phosphopyruvate.

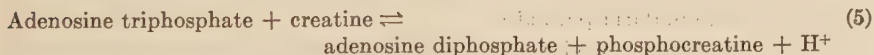
Phosphate Transfer from Phosphopyruvate to Adenosine Diphosphate—Phosphopyruvate is broken down during glycolysis by transfer of its phosphate group to adenylic acid or adenosine diphosphate, to form pyruvate and adenosine triphosphate (25, 26) (Equation 4). This reaction must be studied in the absence of the enzyme adenosinetriphosphatase,



which would hydrolyze the adenosine triphosphate formed. 3-Phosphoglycerate must also be absent; otherwise the adenosine triphosphate would break down by transfer of its phosphate to 3-phosphoglycerate to form 1,3-diphosphoglyceric acid, which spontaneously decomposes to 3-phosphoglycerate and inorganic phosphate (27). An acetone powder of an aqueous rabbit muscle extract (21) was used as the source of the enzyme in studying the transfer of phosphate from phosphopyruvate to adenosine diphosphate; such a powder does not contain an active adenosinetriphosphatase. Fluoride was added to prevent the formation of 3-phosphoglycerate from the phosphopyruvate (23, 28). Samples contained 0.025 M veronal buffer at pH 8.2, 0.005 M MgSO_4 , 0.06 M KCl (29), 0.01 M NaF, 0.005 M phosphopyruvate, 0.0045 M adenosine diphosphate, and dialyzed extract equivalent to 10 mg. of acetone powder, in a total volume of 1.0 cc. In 10 minutes at 37° about 100 γ of phosphopyruvate P per cc. were transferred.

Quinine in concentrations from 0.0005 to 0.002 M caused no inhibition of this phosphate transfer.

Phosphate Transfer from 3-Phosphoglycerate to Creatine—Muscle extract



catalyzes the transfer of phosphate from adenosine triphosphate to creatine (26) (Equation 5). The effect of quinine on the over-all process of phosphate transfer from 3-phosphoglycerate to creatine (the sum of Equations 3 to 5) was investigated. The reaction mixture consisted of 0.025 M veronal buffer at pH 8.7, 0.005 M MgSO_4 , 0.06 M KCl, 0.03 M creatine, 0.00035 M adenosine triphosphate, 0.016 M 3-phosphoglycerate, and dialyzed extract equivalent to 5 mg. of acetone powder of rabbit muscle extract (21), in a total volume of 1.0 cc. The samples were incubated for 15 minutes at 37°. The reaction was followed by determining the increase in phosphocrea-

tine phosphate by the calcium precipitation method of Fiske and Subbarow (30). Balance experiments showed that the increase in phosphocreatine and inorganic phosphate accounted for 94 per cent of the 3-phosphoglycerate phosphate which disappeared, the rest being completely accounted for in phosphopyruvate and adenosine triphosphate. During the experimental period 240 γ of phosphocreatine P and 40 γ of inorganic P per cc. of reaction mixture were formed.

Quinine in concentrations from 0.0005 to 0.002 M caused no inhibition of the transfer of phosphate from 3-phosphoglycerate to creatine.

DISCUSSION

Silverman *et al.* (2) have found that the aerobic and anaerobic glycolysis of chicken erythrocytes parasitized with *Plasmodium gallinaceum* is inhibited about 35 per cent by 0.001 M quinine; *i.e.*, by concentrations of the drug equal to those used in the studies reported in this paper. It would appear from our data that the effects described by Silverman *et al.* are in part due to the action of the drugs on the hexokinase and lactic dehydrogenase of the parasite, since these enzymes catalyze essential steps in the glycolytic process. The inhibition by quinine of the over-all process of lactic acid formation from glycogen by rabbit muscle enzymes is due to the effect of quinine on the enzyme phosphorylase. Likewise, the inhibition of the fermentation of glucose by intact yeast cells observed by Enders and Wieninger (31) may be ascribed to the inhibition of yeast hexokinase by the antimalarial.

In connection with the latter enzyme it should be pointed out that the hexokinase from parasitized red blood cells appears to be less sensitive to quinine than does the hexokinase of yeast and normal chicken erythrocytes, although the effect of atabrine on the enzymes from the three different sources is the same. There is not, as yet, any explanation for this difference in behavior. The other glycolytic enzymes from yeast and muscle show the same sensitivity to quinine and atabrine as the corresponding enzymes of the malaria parasites.

It should be emphasized that the concentrations of antimalarials needed to produce the effects described in this paper are considerably greater than those encountered in the blood and tissue fluids of animals being treated with the drugs. In recent studies on the *in vitro* distribution of quinine between parasitized chicken erythrocytes and a suspending medium, carried out by Dr. Joseph Ceithaml in this laboratory,² it has been found that the ratio of intracellular to extracellular quinine concentrations is not greater than 50, even under circumstances which greatly favor the accumulation of quinine in the red blood cells. The concentration of quinine

² Ceithaml, J. J., and Evans, E. A., Jr., unpublished work.

observed in the red cells of chickens receiving therapeutic doses of the drug is about 10^{-5} M (32). Therefore, unless there occurs a localization of the drugs inside the parasite cell, capable of producing high concentrations of the antimalarials in the immediate vicinity of the sensitive enzymes, it seems unlikely that inhibition of the glycolytic mechanism of the parasite is primarily involved in the therapeutic action of the drugs.

At present it seems more likely that quinine and atabrine inhibit oxidative processes in the metabolism of the malaria parasite, particularly those concerned with the oxidation of lactic and pyruvic acids. The oxygen consumption of the parasite utilizing glucose is inhibited by quinine in concentrations as low as 10^{-5} M (3). With these concentrations there occurs an accumulation of lactic acid, although the rate of glucose utilization is not affected (2). Therefore, it would appear that the effect of the drug is to inhibit the oxidative removal of lactic acid rather than its formation by the process of glycolysis.

SUMMARY

The effects of quinine and atabrine on some of the glycolytic enzymes of the malaria parasite (*Plasmodium gallinaceum*), yeast, and mammalian muscle were studied.

Atabrine inhibited the hexokinase activity and the enzyme lactic dehydrogenase in parasite preparations. Quinine was less effective in both cases. The enzyme 3-phosphoglyceraldehyde dehydrogenase was not affected by either drug.

Both quinine and atabrine inhibited yeast hexokinase.

Quinine inhibited the enzymes phosphorylase and phosphoglucomutase from rabbit muscle. Lactic dehydrogenase from beef heart was strongly inhibited by atabrine but only slightly affected by quinine. The other glycolytic enzymes from rabbit muscle which were studied were not affected by quinine.

The possible significance of these effects in the action of antimalarial drugs is discussed.

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